527 Rec'd PCT/PTO 15 MAY 2000

09/554451

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

REQUEST FOR FILING NATIONAL PHASE OF
PCT APPLICATION UNDER 35 U.S.C. 371 AND 37 CFR 1.494 OR 1.495

To: Asst. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

(Our Deposit Account No. 03-3975

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

Atty Dkt: PM 268066 /C 320.04/G
M# /Client Ref.

From: Pillsbury Madison & Sutro LLP, IP Group:

Date: Monday May 15, 2000

This is a **REQUEST** for **FILING** a PCT/USA National Phase Application based on:

1. International Application	2. International Filing Date	3. Earliest Priority Date Claimed
PCT/GB98/03449	16 November 1998	14 November 1997
⇧ country code	Day MONTH Year	Day MONTH Year (use item 2 if no earlier priority)

4. Measured from the earliest priority date in item 3, this PCT/USA National Phase Application Request is being filed within:

   (a) ☐ 20 months from above item 3 date (b) ☒ 30 months from above item 3 date,

   (c) Therefore, the due date (unextendable) is May 14, 2000

5. Title of Invention IMPROVEMENTS IN OR RELATING TO DETECTION OF MOLECULES IN SAMPLES

6. Inventor(s) MURPHY, Jonathan Paul et al

Applicant herewith submits the following under 35 U.S.C. 371 to effect filing:

7. ☒ Please immediately start national examination procedures (35 U.S.C. 371 (f)).

8. ☒ **A copy of the International Application** as filed (35 U.S.C. 371(c)(2)) is transmitted herewith (file if in English but, if in foreign language, file only if not transmitted to PTO by the International Bureau) including:

   a. ☒ Request;
   b. ☒ Abstract;
   c. 22 pgs. Spec. and Claims;
   d. 4 sheet(s) Drawing which are ☐ informal ☒ formal of size ☒ A4 ☐ 11"

9. ☒ **A copy of the International Application has been transmitted by the International Bureau.**

10. **A translation of the International Application** into English (35 U.S.C. 371(c)(2))
    a. ☐ is transmitted herewith including: (1) ☐ Request; (2) ☐ Abstract;
       (3) ______ pgs. Spec. and Claims;
       (4) ______ sheet(s) Drawing which are:
       ☐ informal ☐ formal of size ☐ A4 ☐ 11"
    b. ☐ is not required, as the application was filed in English.
    c. ☐ is not herewith, but will be filed when required by the forthcoming PTO Missing Requirements Notice per Rule 494(c) if box 4(a) is X'd or Rule 495(c) if box 4(b) is X'd.
    d. ☐ Translation verification attached (not required now).

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11. ☒ **PLEASE AMEND** the specification before its first line by inserting as a separate paragraph:
 a. ☒ --This application is the national phase of international application PCT/GB98/03449 filed November 16, 1998 which designated the U.S.--
 b. ☐ --This application also claims the benefit of U.S. Provisional Application No. 60/ ______, filed ______ .--

12. ☐ Amendments to the claims of the International Application **under PCT Article 19 (35 U.S.C. 371(c)(3)), i.e., before 18th month from first priority date above in item 3, are transmitted herewith (file only if in English) including:**

13. ☒ PCT Article 19 claim amendments (if any) have been transmitted by the International Bureau

14. ☐ Translation of the amendments to the claims **under PCT Article 19 (35 U.S.C. 371(c)(3)), i.e., of claim amendments** made before 18th month, **is attached (required by 20th month from the date in item 3 if box 4(a) above is X'd, or 30th month if box 4(b) is X'd, or else amendments will be considered canceled).**

15. **A declaration of the inventor** (35 U.S.C. 371(c)(4))
 a. ☒ is submitted herewith ☒ Original ☒ Facsimile/Copy
 b. ☐ is not herewith, but will be filed when required by the forthcoming PTO Missing Requirements Notice per Rule 494(c) if box 4(a) is X'd or Rule 495(c) if box 4(b) is X'd.

16. **An International Search Report (ISR):**
 a. Was prepared by ☒ European Patent Office ☐ Japanese Patent Office ☐ Other
 b. ☒ has been transmitted by the international Bureau to PTO.
 c. ☒ copy herewith (2 pg(s).) ☒ plus Annex of family members (1 pg(s).).

17. **International Preliminary Examination Report** (IPER):
 a. ☒ has been transmitted (if this letter is filed after 28 months from date in item 3) in English by the International Bureau with Annexes (if any) in original language.
 b. ☒ copy herewith in English.
 c.1 ☐ IPER Annex(es) in original language ("Annexes" are amendments made to claims/spec/drawings during Examination) including attached amended:
 c.2 ☐ Specification/claim pages #_____ claims #
 Dwg Sheets #
 d. ☐ Translation of Annex(es) to IPER **(required by 30th month due date, or else annexed amendments will be considered canceled).**

18. **Information Disclosure Statement** including:
 a. ☒ Attached Form PTO-1449 listing documents
 b. ☒ Attached copies of documents listed on Form PTO-1449
 c. ☒ A concise explanation of relevance of ISR references is given in the ISR.

19. ☒ **Assignment** document and Cover Sheet for recording are attached. Please mail the recorded assignment document back to the person whose signature, name and address appear at the end of this letter.

20. ☐ Copy of Power to IA agent.

21. ☐ **Drawings** (complete only if 8d or 10a(4) not completed): ___ sheet(s) per set: ☐ 1 set informal; ☐ Formal of size ☐ A4 ☐ 11"

22. ☒ 1 (No.) **Verified Statement(s)** establishing "small entity" status under Rules 9 & 27

23. **Priority** is hereby claimed under 35 U.S.C. 119/365 based on the priority claim and the certified copy, both filed in the International Application during the international stage based on the filing in (country) GREAT BRITAIN of:

	Application No.	Filing Date		Application No.	Filing Date
(1)	9723955.2	Nov. 14, 1997	(2)		
(3)			(4)		
(5)			(6)		

 a. ☒ See Form PCT/IB/304 sent to US/DO with copy of priority documents. If copy has not been received, please proceed promptly to obtain same from the IB.
 b. ☒ Copy of Form PCT/IB/304 attached.

RE: USA National Filing of PCT/GB98/03449

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24. Attached: 6 pages of Sequence Listing

25. **Preliminary Amendment:** ATTACHED

25.5 Per Item 17.c2, **cancel original** pages #_____, claims #_____, Drawing Sheets #

26. **Calculation of the U.S. National Fee (35 U.S.C. 371 (c)(1)) and other fees is as follows:**
Based on amended claim(s) per above item(s) ☐ 12, ☐ 14, ☐ 17, ☒ 25, ☐ 25.5 (hilite)

Total Effective Claims	24	minus 20 =	4	x $18/$9	=	$36	966/967
Independent Claims	6	minus 3 =	3	x $78/$39	=	$117	964/965
If any proper (ignore improper) Multiple Dependent claim is present,				add$260/$130		+0	968/969

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(4)): ➔➔ **BASIC FEE REQUIRED, NOW** ➔➔➔➔

A. If country code letters in item 1 are **not** "US","BR","BB","TT","MX","IL" "NZ", "IN" or "ZA"

See item 16 re:
1. Search Report was not prepared by EPO or JPO --------- add$970/$485 — 960/961
2. Search Report was prepared by EPO or JPO ------------ add$840/$420 +420 — 970/971

SKIP B, C, D AND E UNLESS country code letters in item 1 are "US","BR","BB","TT","MX","IL", "NZ", "IN" or "ZA"

(X) (only) (one) (of) (these) (4) (boxes)

➔ ☐ B. If USPTO did not issue both International Search Report (ISR) and (if box 4(b) above is X'd) the International Examination Report (IPER), ------------------- add$970/$485 +0 — 960/961

➔ ☐ C. If USPTO issued ISR but not IPER (or box 4(a) above is X'd), ------------------------------------ add$690/$345 +0 — 958/959

➔ ☐ D. If USPTO issued IPER but IPER Sec. V boxes not all 3 YES, ------------------------------------ add$670/$335 +0 — 956/957

➔ ☐ E. If international preliminary examination fee was paid to USPTO and Rules 492(a)(4) and 496(b) satisfied (IPER Sec. V all 3 boxes YES for all claims), ------------- add $96/$48 +0 — 962/963

27. **SUBTOTAL =** $573

28. If Assignment box 19 above is X'd, add Assignment Recording fee of ----$40 +40 (581)

29. Attached is a check to cover the ------------------------ **TOTAL FEES** $613

Our Deposit Account No. 03-3975
Our Order No. 41301 (C#) | 268066 (M#)

CHARGE STATEMENT: The Commissioner is hereby authorized to charge any fee specifically authorized hereafter, or any missing or insufficient fee(s) filed, or asserted to be filed, or which should have been filed herewith or concerning any paper filed hereafter, and which may be required under Rules 16-18 and 492 (missing or insufficient fee only) now or hereafter relative to this application and the resulting Official document under Rule 20, or credit any overpayment, to our Account/Order Nos. shown above for which purpose a duplicate copy of this sheet is attached.
This CHARGE STATEMENT does not authorize charge of the issue fee until/unless an issue fee transmittal form is filed

Pillsbury Madison & Sutro LLP
Intellectual Property Group

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By Atty: **Paul N. Kokulis** **Reg. No.** **16773**

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NOTE: File in duplicate with 2 postcard receipts (PAT-103) & attachments.

Fax from : 01223 324353 12/05/00 15:03 Pg: 2

MAY-11-2000 18:00

Inventor(s): MURPHY, Jonathan Paul et al PM&S - WDC 202 822 0944

Appln No.: / or Patent No.: 202 822

Filed: May 15, 2000 or Issued: 288086/MJI/JGB/C320.04/0

Title: IMPROVEMENTS IN OR RELATING TO DETECTION OF MOLECULES IN SAMPLES MII / Client Ref.

SMALL ENTITY STATEMENT CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(d) and 1.27 (c)) - SMALL BUSINESS CONCERN

I hereby state that I am

☐ the owner of the small business concern identified below:

☐ an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN

ADDRESS OF CONCERN

I hereby state that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.12, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby state that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention entitled: Improvements in or relating to detection of molecules in samples by inventor(s) MURPHY, Jonathan P. et al described in

☐ the specification filed herewith.

☐ Application No. 0 /_____, filed May 15, 2000

☐ Patent No. _____, issued

If the rights held by the above identified small business concern are not exclusive, each small entity individual, concern or organization having rights to the invention is listed in (A) and (B) below and no rights to the invention are held by any person, other than the inventor, who could not qualify under 37 CFR 1.9(c) as an independent inventor if that person had made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

(A) FULL NAME of assignee/licensee/grantee/conveyee*

ADDRESS

☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

(B) FULL NAME of assignee/licensee/grantee/conveyee*

ADDRESS

☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

*NOTE: Separate statement is required from each person, concern or organization named in (A) and (B) above having rights to the invention, averring to his/her/its status as a small entity. (37 CFR 1.27)

I acknowledge the duty to file, in this case, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

NAME OF PERSON SIGNING DR. JONATHAN MURPHY

TITLE OF PERSON OTHER THAN OWNER COMMERCIAL DEVELOPMENT

ADDRESS OF PERSON SIGNING 37, BISHOPS ORCHARD, EAST HAGBOURNE, OXFORDSHIRE. UK. OX11 9JS

SIGNATURE J. Murphy DATE 15/5/00

09/554451

526 Rec'd PCT/PTO 15 MAY 2000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

MURPHY ET AL

Serial No.: Group Art Unit:

Filed: May 15, 2000 Examiner:

Title: IMPROVEMENTS IN OR RELATING TO DETECTION OF MOLECULES IN SAMPLES

May 15, 2000

PRELIMINARY AMENDMENT

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

Please amend the above application as follows:

IN THE CLAIMS

Claim 3, line 1, delete "or 2".

Claim 4, line 1, delete "any one of claims 1, 2 or 3" and insert -- claim 1 --.

Claims 5, 6 and 7, line 1 of each, delete "any one of the preceding claims" and insert -- claim 1 --.

Claim 9, line 1, delete "or 8".

Claims 10, 11 and 12, line 1 of each, delete "any one of the preceding claims" and insert -- claim 1 --.

Claim 15, line 1, delete "or 14".

Claim 16, line 1, delete "any one of claims 13, 14 or 15" and insert -- claim 13 --.

Claim 17, line 1, delete "any one of claims 13-16" and insert -- claim 13 --.

Claim 18, line 1, delete "any one of claims 13-17" and insert -- claim 13 --.

Claim 22, lines 1-2, delete "any one of claims 19, or 21" and insert -- claim 19 --.

Cancel claims 25 and 26.

REMARKS

The foregoing amendments are being made to improve the dependency and form of the claims and to reduce the number of claims for consideration.

Favorable action is requested.

Respectfully submitted,

CUSHMAN DARBY & CUSHMAN
Intellectual Property Group of
Pillsbury Madison & Sutro, LLP

By ______________________
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09/554451
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APPLICATION UNDER UNITED STATES PATENT LAWS

Atty. Dkt. No. PM 268066/C 320.04/G
(M#)

Invention: IMPROVEMENTS IN OR RELATING TO DETECTION OF MOLECULES IN SAMPLES

Inventor (s): MURPHY, Jonathan Paul
ATKINSON, Anthony

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This is a:

- ☐ Provisional Application
- ☐ Regular Utility Application
- ☐ Continuing Application
- ☒ PCT National Phase Application
- ☐ Design Application
- ☐ Reissue Application
- ☐ Plant Application
- ☐ Substitute Specification
  Sub. Spec Filed ________
  in App. No. ____ / ____
- ☐ Marked up Specification re
  Sub. Spec. filed ________
  In App. No ____ / ____

SPECIFICATION

 4/PRTS 09/554451
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PCT/GB98/03449

Title: Improvements in or Relating to Detection of Molecules in Samples

Field of the Invention

This invention relates to tagged molecules (distinguishable from untagged, but otherwise identical, molecules), methods of preparing tagged molecules, nucleic acid sequences and constructs encoding tagged molcules, and a method of distinguishing between tagged and untagged (but otherwise identical) molecules.

In particular, the present invention relates to a method of tagging a protein with a therapeutically acceptable tag which enables detection of the tagged protein administered exogenously to humans, bovines or other animals where the same (but untagged) protein is produced endogenously, and a method of detecting and differentiating the tagged protein over the endogenous protein. In particular, the method is suitable for application to human growth hormone (hGH), to enable differential detection of exogenously administered hGH in humans, for example, to determine whether hGH is being administered unlawfully for its performance enhancing effects.

Background of the Invention

Previously, the usual method of differentiating exogenously administered protein from the endogenous one has been to tag the exogenous protein with radioactive labels. Because of the hazards of radioactivity, radioactively tagged proteins are administered to patients over short periods of time in controlled conditions and under medical supervision. Further, radioactive labels are not therapeutically acceptable since they are intrusive to the biological system in which such tagged proteins are administered. Other tagging methods tend to alter the biological function of the protein molecule and therefore, are no longer suitable for therapeutic use. Such prior art tagging methods are therefore limited to controlled research uses and do not have widespread cost effective commercial applications.

Some amino acids, for example tryptophan (W) and tyrosine (Y) in particular, are natural fluorophores, which fluoresce when appropriately stimulated. This fluorescence can be detected and measured with standard prior art fluorescence detection techniques. Proteins

which contain such fluorophores in their amino acid sequence may also fluoresce when appropriately stimulated. The level of fluorescence can be crudely related to the number of fluorophores in the protein. The fluorescent yield of any fluorophore is sensitive to its local environment such that, for example, there may be a difference between its fluorescence in an aqueous and a hydrophobic environment. Waldman *et al* (1987 Biochem. Biophys. Acta *931*, 66-71; 1988 Biochem. Biophys Res. Comm. *150* (2), 752-759), Corinne (1991 Biochemistry *30*, 1028-1036) and others have exploited this property to perform *in vitro* laboratory studies on conformational and structural changes of lactate dehydrogenase when, for example, substrate binding occurs. Waldman and Corrine have mutated lactate dehydrogenase to incorporate tryptophan residues at the substrate binding site. This technique is restricted to use as a research tool for conformational and structural studies of proteins *in vitro*, since often the full biological activity or structural conformation of the native protein is lost. Thus, such modified proteins are no longer suitable for therapeutic purposes and there is no disclosure or suggestion of pharmaceutical compositions comprising the mutated protein. Moreover, there is no disclosure or suggestion in the prior art that such mutations could form the basis for a method of distinguishing the altered compound from the naturally occurring compound.

WO 94/10200 discloses and is concerned with amino acid substitutions in somatotropin (i.e. Growth Hormone) which provide increased conformational and chemical stability.

There is no suggestion in WO 94/10200 that modifications can be made to Growth Hormone for the purpose of distinguishing between endogenous Growth Hormone present in a subject and exogenous Growth Hormone administered to the subject. A number of amino acid substitutions in somatotropin are disclosed or suggested in WO 94/10200 which, because of the natural fluorophore activity of the amino acid residues tryptophan and tyrosine (discussed above), result in a somatotropin molecule having an altered fluorescence activity relative to the wild type, unsubstituted molecule. Such substitutions include the following:

G40→Y (i.e. glycine substituted by tyrosine at residue number 40); F52→Y; W86→F, Y, L, I or V; F103→Y; I137→Y;

A reliable method for differentiating and detecting exogenously administered hGH is particularly desirable when attempting to monitor the pharmacokinetics and/or

pharmacodynamics of hGH, or to detect its unlawful administration by athletes and others who illicitly use hGH for improving their performance. Presently, standard detection methods (e.g. HPLC, ELISA), are used for measuring the total amount of hGH in an athletes' blood or urine samples, and by subtracting the expected levels referenced to the general population, estimations of elevated hGH levels can be made. However, as levels vary considerably between individuals, and exogenous levels fall rapidly with time, this is a very crude measurement. In addition, as the performance enhancing effects last much longer than the detectable transient elevated levels of hGH in these samples, unless samples are taken shortly after administration the technique does not give indisputable proof that exogenous hGH has or has not been used.

The present invention seeks to alleviate the above mentioned problems by tagging or modifying a protein (such as hGH) with a therapeutically acceptable tag which can be detected simply and can be differentiated from the endogenous protein present in a sample of cells, blood, urine or other body fluid. The invention has little or no effect on the biological activity of the protein, such that the modified protein can be administered therapeutically in the same manner as the unmodified protein. Thus, the modified or tagged protein can be safely prescribed by physicians for existing or new therapeutic purposes, and also economically manufactured commercially at substantially the same cost as the untagged protein.

A further advantage of the present invention is that although levels of the exogenous protein may drop rapidly after administration, the specificity for the tagged protein and high sensitivity of the detection method allow detection long after the exogenous protein has been administered. Thus, an abuser cannot claim abnormally elevated production of the endogenous protein, and unlawful use of the tagged protein can be detected. Additionally, the present invention allows the pharmacokinetics and/or pharmacodynamics of the tagged exogenous protein to be detected and monitored.

Therefore, it is an object of the present invention to provide a method for tagging proteins which method enables detection of the exogenous tagged protein over any endogenous polypeptide which may be present in a sample (e.g. such as blood or urine) taken from, for example, a human subject (e.g. an athlete) or other mammalian subject (e.g. domesticated

farm livestock).

It is another object of the present invention to provide a modified polypeptide molecule, such as hGH, tagged in a manner which is therapeutically acceptable. Further, the tagging method of the present invention enables the biological activity *per se* of a protein to remain substantially unaltered such that the therapeutic efficacy is maintained and the protein can be administered in a manner identical to or similar with the unmodified protein.

A further specific object of the present invention is to provide a modified hGH molecule substituted with tryptophans at strategic positions in the native hGH sequence.

Summary of the Invention

In a first aspect, the invention provides a method of detecting the presence in a sample of a polypeptide exogenously administered to a mammalian subject from whom the sample is obtained, and distinguishing between such an exogenously administered polypeptide and a naturally-occurring endogenous polypeptide present in the sample; the method comprising obtaining a sample from the subject; and subjecting the sample to analysis of fluorescence at a suitable wavelength; wherein the exogenously administered is tagged with a greater or lesser amount of fluorescence activity, relative to the untagged endogenous polypeptide, at the wavelength(s) analysed.

In a second aspect, the invention provides a composition for administration to a mammalian subject, the composition comprising a polypeptide and a physiologically acceptable carrier substance, characterised in that the polypeptide is tagged with a greater or lesser amount of fluorescent activity relative to an untagged polypeptide endogenously present in the subject, the tagged molecule thereby being distinguishable from the untagged molecule by analysis of the fluorescence characteristics of the respective molecules, excluding those compositions in which the tagged molecule is Growth Hormone and wherein the fluorescent tagging consists solely of one or more of the following substitutions in the tagged Growth Hormone: G40 → Y; F52 → Y; W86 → F, Y, L, I or V; F103 → Y; and I137 → Y.

The tagged molecule is a polypeptide, which may typically be administered to a mammalian subject to exert a beneficial effect (e.g. for clinical or veterinary reasons, or for reasons of animal husbandry). The mammalian subject will generally be human. but may also be a

domesticated animal, especially a farm animal such as a bovine, porcine or ovine animal. The tagged molecule will generally therefore be a therapeutic polypeptide (i.e. comprises five or more amino acid residues and has a desirable effect on the subject, with little or no undesired side effect, when administered in an appropriate dose) and will possess the same biological activity as, and normally be substantially identical (except for the tagging) to, a naturally-occurring polypeptide present in the subject, although where the tagged molecule is a recombinant polypeptide it may have additional slight differences relative to the naturally occurring polypeptide (e.g. to increase activity, or to increase stability, e.g. as taught in WO 94/10200). (The "biological activity" of the molecule is that activity by which the molecule exerts its beneficial effect on the subject e.g. stimulation of growth in the case of GH; or stimulation of erythrocyte production in the case of EPO.)

The molecule may be, for example, a pharmaceutical. A particularly preferred molecule is a mammalian growth hormone, especially human growth hormone (hGH), bovine growth hormone (bGH), or porcine growth hormone (pGH); or calcitonin; or erythropoietin (EPO). Accordingly it is preferred that any fluorophores present in the tagged molecule: (a) do not have any significant effect on the biological activity of the molecule; and (b) are essentially non-toxic (that is, any fluorophores present will not cause the tagged molecule to exhibit any toxicity for the subject when the molecule is administered at normal therapeutic doses). Accordingly, tryptophan or tyrosine and closely-related compounds are preferred fluorophores for use in tagged molecules in accordance with the invention. These have the additional advantage of being readily incorporated into polypeptide molecules.

Advantageously, the tagged molecule is either deficient in, or comprises additional, fluorescent entities (fluorophores) relative to the untagged molecule. The tagging may therefore be "positive" (in which the tagged molecule comprises additional fluorophores) or "negative" (where the tagged molecule is deficient in fluorophores relative to the untagged molecule).

As explained above, the naturally occurring amino acid residues tryptophan (W) and, to a lesser extent, tyrosine (Y), possess natural fluorophore activity. Thus, if an "untagged" polypeptide comprises one or more tryptophan and/or tyrosine residues it may be fluorescent. Thus a tagged molecule, in accordance with the invention, may be distinguishable from an

untagged molecule by having additional fluorophores (especially if the untagged polypeptide comprises no, or very few, tryptophan or tyrosine residues and thus possesses no, or very little, intrinsic fluorescence). Alternatively, where the untagged molecule comprises a fluorophore (especially a plurality of fluorophores), the tagged molecule may be distinguishable by having fewer fluorophores than the untagged molecule.

Preferably, the tagged molecule comprises additional fluorophores present in amino acid residues or other compounds which are capable of forming a peptide bond, and thus are capable of being covalently incorporated into a polypeptide, either internally during synthesis of the polypeptide, and/or at the C-terminal after synthesis of the bulk of the polypeptide.

Conveniently the fluorophores additionally present in (or absent from) the tagged molecule (relative to the untagged molecule) are tyrosine and/or tryptophan residues, or a synthetic amino acid derivative wherein a fluorophore is covalently joined to an "amino acid" backbone, the synthetic derivative having the general formula

$$\begin{array}{c} R_2 \\ | \\ NH_2\text{-}C\text{-}COOH, \\ | \\ R_1 \end{array}$$

wherein R_1 comprises the fluorophore and R_2 is H, OH, halide or lower alkyl (C_1 to C_3, substituted or unsubstituted). The fluorophore R_1 may be a fluorophore which is present in a naturally occurring amino acid residue (e.g. the aromatic groups of tryptophan or tyrosine) or may be some other fluorophore (typically comprising a delocalised electron system, such as in an aromatic or heterocyclic ring). Such synthetic amino acid derivatives are already known in the art or can readily be prepared using standard organic chemistry techniques.

As a less preferable alternative to the tagged molecule comprising a different number of fluorophores (relative to the untagged molecule), the tagged molecule may comprise the fluorophores at different positions - the immediate chemical environment can affect the level of fluorescence of a fluorophore. Accordingly, the tagged molecule may not have a different number of fluorophores relative to the untagged molecule, but they may be of different

fluorescent activities and/or be differently disposed within the molecule so as to affect their fluorescence.

Where the tagged molecule is a polypeptide, tagging is conveniently accomplished by substituting a non-fluorescent amino acid present in the untagged molecule for an amino acid residue comprising a fluorophore (such as tryptophan, tryosine or a synthetic amino acid derivative), so as to increase the fluorescence of the tagged molecule relative to the untagged molecule.

With the benefit of the teaching of the present specification, and with the benefit of information otherwise readily available as common general knowledge, the person skilled in the art can, by routine trial and error, find appropriate amino acid residues which can be substituted, without substantially affecting the biological activity of the molecule. Conveniently, phenylalanine residues (F) or tyrosine residues (Y) can be replaced with tryptophan residues (W), which exhibit far greater fluorescence activity. Such substitutions are "conservative" and thus tend not to have any significant effect on the biological properties of a polypeptide. Further guidance for the person skilled in the art is given in the example below, which utilises principles which are generally applicable to any biologically active polypeptide.

The composition will normally comprise an effective amount of the tagged molecule, such that the biological activity thereof produces a demonstrable effect when administered to the subject. An "effective amount" is the amount of tagged molecule which results in the desired biological effect in the mammalian subject to which the composition is administered. The desired effect will, of course, depend on the identity of the tagged molecule: where the tagged molecule is EPO, for example, the desired effect is an increase in the number of erythrocytes per unit volume of blood in the subject. In some embodiments the composition will be essentially sterile, and suitable for delivery by means of injection (e.g. by trans-dermal, intravenous, intramuscular or subcutaneous routes). In other embodiments the composition will be in the form of a tablet, pill or capsule (e.g. enteric-coated capsules for slow release) for oral consumption.

Administration of the compositions of the invention into a mammalian subject may be performed according to known methods using any route effective to deliver the required

dosage to the subject. Modes of administration include those typically encountered for the species of choice. Because proteins in general are susceptible to degradation in the digestive system, injection is preferred via an intramuscular, transdermal or subcutaneous route. The use of sustained or prolonged release formulations or implants are also suitable modes. Generally, injection of a sustained release formulation is preferred.

The effective dosage range depends on the species, age, weight, and general health of the mammalian subject. These and other parameters which are needed to determine the effective dosage range for a given mammal are well within the purview of one skilled in the art. For instance, in bovines the effective amount of bovine GH (whether tagged or untagged) is in the range of 1.0 to 200 milligrams per animal per day. In pigs, for instance the effective amount of porcine GH is about 60 μg/kg/day.

The physiologically acceptable carrier may be a sterile liquid diluent where the composition is injected (e.g. saline, phosphate-buffered saline, or other aqueous buffer preparation). Where the composition is to be administered orally or transdermally, the carrier may be calcium carbonate, calcium sulphate or other substantially inert solid. Transdermal delivery by means of a needleless injection device may generally be preferred.

Methods of performing the fluorescence analysis may be entirely conventional and well known to those skilled in the art (e.g. spectrofluorimetry). The choice of method will depend in part on the manner in which the exogenous substance is tagged, and the characteristics of the fluorophore (if any) employed in the tagged molecule. For example, where the tagged molecule comprises fewer or more tryptophan residues than the untagged molecule, fluorescence analysis will typically be performed at about 297nm excitation.

Advantageously the sample is subjected to processing, prior to fluorescence analysis, to enrich or purify the endogenous and (if present) exogenous molecules in the sample. This improves the signal-to-noise ratio. Various methods of enrichment or purification may be employed, using one or more of the following techniques: centrifugation; HPLC; FPLC; affinity chromatography; immunoaffinity chromatography; heat treatment at 50-55°C for ten minutes (this is particularly appropriate for purification of growth hormone, which is relatively heat-stable - contaminating proteins will tend to be denatured, aggregate and precipitate, and so can be simply removed by centrifugation whilst the undenatured growth

hormone stays in solution); all of which are well known to those skilled in the art. The preferred method may depend, at least in part, on the identity of the endogenous and exogenous molecules.

The method defined immediately above is extremely useful in detecting the presence of exogenously administered molecules used illicitly by cyclists, athletes and others to improve performance. Very often, such molecules occur naturally (e.g. EPO, hGH, and the like) and are endogenous to the athlete's body, such that proving illicit use of performance-enhancing substances is very difficult. However, with the benefit of the present invention, such substances can be tagged, and thus made distinguishable over endogenous molecules synthesised naturally in the athlete's body.

Additionally the invention can be used to monitor the persistence of substances administered to the body. For example, the pharmacokinetics and/or pharmacodynamics of various drugs can readily be monitored by comparing fluorescence activities at different time points - this is particularly useful where the tagged drug is otherwise identical to an endogenous compound.

In a preferred embodiment, the tagged molecule is a polypeptide prepared using recombinant DNA technology. In such embodiments the method may additionally comprise the preparation of a nucleic acid sequence encoding the tagged molecule, the sequence being mutated relative to the wild type sequence encoding the untagged molecule. Typically the nucleic acid sequence encoding the tagged polypeptide comprises nucleotide substitutions (relative to the wild type sequence) so as to direct the expression of a polypeptide having one or more tryptophan residues not present in the untagged molecule or, less preferably, directing the expression of a polypeptide having fewer tryptophan residues than in the untagged molecule.

The nucleic acid sequence encoding the tagged molecule may be prepared, for example, by mutation of the wild-type sequence (e.g. by site-directed mutagenesis), by polymerase chain reaction (PCR), or by *de novo* synthesis (e.g. using an automated DNA synthesiser). All of these techniques are familiar and well-known to those skilled in the art and/or are readily obtained by reference to standard texts in the field (e.g. Sambrook *et al*, "Molecular Cloning, A Laboratory Manual" Cold Spring Harbor Laboratory Press, 1989).

Where the subject is a human, the sample may conveniently be a sample of body fluids, such as a blood, sweat, semen, urine, or saliva sample. Less preferably the sample may be a tissue sample comprising cells (e.g. skin scrapings from the buccal cavity, hair or the like). Where the subject is a domesticated farm animal, the sample may be taken from the animal before or after slaughter. Samples taken after slaughter conveniently include muscle tissue or other solid tissues taken from the carcass.

In another aspect of the present invention there is provided a tagged GH molecule comprising a tryptophan residue substituted for a phenylalanine residue present in a naturally-occurring molecule. In one embodiment, tryptophan is substituted at positions F31 and/or F97 in the amino acid sequence.

In a preferred embodiment, the tagged growth hormone comprises a tryptophan residue at one or more of positions 10, 31, 97, 160 or 176 (of which tryptophan residues at positions 31 and/or 97 are especially preferred). The tagged growth hormone molecule is preferably tagged hGH.

According to a still further aspect of the present invention there is provided a nucleic acid expression vector comprising substantially nucleotides 114-695 of the nucleic acid sequence shown in Figure 2. The CPG_2 signal sequence (nucleotides 39-113) is intended to direct the encoded polypeptide product to the bacterial periplasm - those skilled in the art will appreciate that the CPG_2 signal does not form an essential part of the vector, but is useful for expression in prokaryotes. Other signal sequences are well known to those skilled in the art and could be substituted for the CPG_2 signal sequence if desired. Thus the expression vector may be designed to cause expression in eukaryotes (e.g. mammalian tissue culture, fungal or yeast cultures) or in prokaryotes (bacterial cultures). In a particular embodiment the expression vector is a prokaryotic expression system, preferably comprising the vector pMTLhGHm described below.

The invention will now be described by way of illustrative examples and with reference to the accompanying drawings, in which:-

Figure 1 shows the primary amino acid sequence (Seq. ID No. 1) of native hGH protein;

Figure 2 shows a nucleic acid sequence (Seq. ID No. 2) encoding a tagged hGH molecule

for use in the method of present invention:

Figure 3 shows the primary amino acid sequence (Seq. ID No. 3) of the tagged hGH molecule encoded by the nucleic acid sequence of Figure 2;

Figure 4 is a schematic representation of the nucleic acid construct pMTLhGHm used to express a tagged polypeptide in accordance with the invention;

Figure 5 shows the amino acid sequence of human calcitonin (Seq. ID No. 4) - the sequence is shown in the orientation N terminal→C terminal, but the C terminal residue includes a naturally occurring amide group (as a post-translational modification);

Figure 6 shows the amino acid sequence of human growth hormone releasing factor (HGHRF) (Seq. ID No. 5) - the sequence is shown in the orientation N terminal→C terminal, but the C terminal residue includes a naturally occurring amide group (as a post-translational modification);

Figures 7A and 7B show the amino acid sequence of the A and B chains respectively of human insulin (Seq. ID Nos. 6 and 7);

Figure 8 shows the amino acid sequence of human Erythropoietin (Seq. ID No. 8); and

Figure 9 shows the amino acid sequence of human Interleukin 2 (Seq. ID No. 9).

Example 1 - Construction of an enhanced fluorescent form of hGH.

Amino acids can be generally classified into 4 main classes depending on their R groups: (1) non-polar or hydrophobic R groups; (2) neutral (uncharged) polar R groups; ((3) positively charged R groups; and (4) negatively charged R groups.

Although within any single class there is considerable variation in the size, shape, and properties of the R groups, certain amino acids show similar properties and can often be substituted without dramatically changing the protein conformation or biological activity. It has been suggested that there is a 70-80% chance of attaining a mutated protein with unchanged biological properties, by replacing any one phenylalanine (F) or tyrosine (Y) residue by tryptophan (W). However, it is not always possible accurately to predict the actual effect of such substitutions on the conformation and/or biological activity of a protein.

The extent of the effect or sensitivity of the protein to such substitution(s), will depend on the function of the target amino acid residue which is to be substituted. If the target amino acid is involved in catalysis or interacts with another residue, the protein will be sensitive to substitution. However, if the target amino acid is a scaffolding residue, the protein will be less sensitive to substitution by an amino acid with a similar R group.

In the native hGH amino acid sequence, there are twelve F, eight Y and one W residues (see Figure 1). In the method described below, techniques available in the art are used to establish which of the F and Y residues will have the least effect on the structure and biological activity of hGH, if substituted with W.

Determination:

Using the available 3D X-ray structures of hGH alone and hGH complexed with its receptor (both obtained from the Brookhaven data base) the positions of twenty potential substitutions were analysed in order to filter out the sensitive substitutions by Environmental Filtering which involves:-

1) Eliminating residues close to the surface of the protein:-

The inventors find that substitutions at surface sites should be avoided since the added hydrophobic character of the tryptophan residue sometimes gives rise to increased protein aggregation. Further, modifications to residues at the surface of a polypeptide are generally undesirable as they may (i) interfere with binding activity of the protein; and (ii) are more likely to create a new epitope which may be recognised as foreign by the immune system of the recipient. The following residues were shown to be surface residues and were therefore regarded as poor candidates for substitution in hGH:- F1; F25; Y35; Y42; F44; F54; F92; Y103; Y111; Y143; F146; F191.

2) Eliminating residues close to inter-protein surfaces: -

The remaining eight residues were determined to be buried in the protein conformational structure. Y164 and Y28 were determined to have close proximity to the hGH receptor glutamate residue and therefore likely to be critical in the interaction between hGH and its receptor. Thus, residues Y164 and Y28 are poor candidates for substitution.

3) Eliminating residues close to W86:-

It is known that fluorophores which are in close proximity to each other can interact through internal energy transfer (a blue shift in emission) thereby quenching the individual fluorescence of each fluorophore. F166 was determined to be in close proximity to W86, the single naturally occurring fluorophore in the native hGH protein. In order to avoid such potential quenching effects which could reduce the desired effect of fluorescence enhancement of the mutated protein, F166 was regarded as a poor candidate for substitution.

From the above analysis the inventors determined that the best candidates for W substitutions are: F31, F97, F10, F176 and/or Y160. Further analysis of the environmental position of these five residues within the native hGH revealed suitability for W substitution (see Table 1). In the example given below, F31 and F97 were selected for construction of the modified protein, and the other remaining residues are potentially suitable candidates also. It will be appreciated that other amino acids at suitable sites may be similarly substituted, and that, whilst substitution of F or Y residues is preferred, the invention is not so limited.

Table 1 shows the five tryptophan substitutions predicted as least likely to alter the hGH protein conformation and therefore least likely to affect biological activity.

RESIDUE	ENVIRONMENT	DISTANCE TO RECEPTOR SURFACE
F31	Hydrophobic cluster just below surface. Between helices.	Great
F97	Deep in a surface cleft	Great
F10	Hydrophobic surface slot	Adequately remote
F176	Buried, but close to W86	Adequately remote
Y160	Hydrophobic cluster just below the surface	About 0.6nm from receptor surface

The principles described above (i.e. avoiding substitution of residues which are surface-exposed or near functional sites such as active or allosteric sites of enzymes or receptor-

binding sites of ligands; and avoiding substitution of residues near other fluorescent residues) can be used to identify phenylalanine residues in any other biologically active molecule which are suitable for substitution by tryptophan, thereby allowing any desired polypeptide to be tagged, relative to a naturally-occurring endogenous polypeptide, so that the method of the invention can be applied very generally.

Example 2 - Construction of gene by substituting W for F31 and F97.

Optimised gene sequence: Using the empirically observed codon utilisation bias for highly expressed *E. coli* genes, the known DNA sequence coding for native hGH was re-designed incorporating, where possible, this *E. coli* codon bias whilst ensuring retention of the original translated protein sequence. The two substitutions (W31 and W97) were then incorporated into this optimised *E. coli* gene sequence. It will be appreciated by those skilled in the art that optimisation of codon bias for *E. coli* may not be desirable if the sequence is to be expressed in a host other than *E. coli*.

Figure 2 shows the nucleotide sequence used to encode the modified hGH. To facilitate expression and subsequent purification, the hGH coding sequence is preceded by a 75bp fragment of DNA derived from the carboxypeptidase G_2 (CPG_2) gene encoding the twenty-five amino acid signal peptide of CPG_2 (Minton *et al*, 1985 Gene *31*, 31-38). The CPG_2 signal peptide directs the expressed protein to the periplasm where the CPG_2 signal sequence is enzymatically cleaved, releasing the authentic hGH protein into the periplasm. Those skilled in the art will appreciate that the CPG_2 signal peptide sequence could be replaced with any one of a large number of functionally equivalent signal sequences from other sources, without substantially affecting the nature of the construct.

The amino acid sequence of the tagged hGH encoded by the nucleic acid sequence is shown in Figure 3; the tryptophan substitutions at positions 31 and 97 are shown in bold type.

Construction of the synthetic gene: This synthetic gene was constructed by standard chemical procedures (Wosnick *et al*, 1987 Gene *60* (1), 115-127) using double-stranded annealed pairs of 60-100 bp oligonucleotides with appropriately compatible sticky ends.

Cloning: Using standard techniques, the synthesised gene was restricted with *Nde* I and *Xho* I and cloned into the identical restriction sites of pMTL1015 (Chambers *et al*, 1986 Gene

68(1), 139-149) to produce pMTL hGHm (illustrated schematically in Figure 4). This vector directs expression of the synthetic gene under the control of the *mdh* promoter (Alldread *et al*, 1992 Gene 114(1), 139-143), and carries a selectable tetracycline resistance gene (Tc^R). Those skilled in the art will appreciate that the *mdh* promoter could be replaced with any one of a large number of functionally equivalent promoters from other sources, without substantially affecting the nature of the construct.

The new construct, pMTLhGHm, was transformed and subsequently expressed in an appropriate strain of *E. coli* (K-12 strain RV308, ATCC 31608) using standard procedures.

Production and Purification: The modified hGH protein (hGHf) may be produced in an industrial scale fermenter by methods well known to those skilled in the art. For example, a transformed *E. coli* culture containing pMTLhGHm may be grown up in aqueous media in a steel or other fermentation vessel conventionally aerated and agitated, in aqueous media at e.g. about 28-37°C and near neutral pH, supplied with appropriate nutrients such as glycerol, nitrogen sources such as ammonium sulphate, potassium sources such as potassium phosphate, trace elements, magnesium sulphate and the like. The plasmid pMTLhGHm carries tetracycline resistance as a selectable characteristic, so that selection pressure (i.e. inclusion in the medium of tetracycline at 12.5μg/ml) may be imposed to discourage competitive growth from wild-type organisms which lack the resistance characteristic (e.g. due to "segregation" of the plasmid during growth of the culture).

Upon completion of fermentation the cell suspension is centrifuged or the cellular solids otherwise collected from the broth and then lysed by physical or chemical means. Cellular debris is removed from supernatant and soluble hGHf isolated and purified.

HGHf may be purified from cell extracts using one or more of the following techniques: (i) polyethyleneimine fractionation; (ii) gel filtration chromatography on Sephacryl S-200; (iii) ion exchange chromatography on ToyoPearl Super Q 650m or CM Sephadex; (iv) hydrophobic chromatography using Phenyl-Sepharose; (v) ammonium sulphate and/or pH fractionation; (vi) selective heat enrichment; and (vii) affinity chromatography using antibody resins prepared from anti-hGH IgG isolated from immunosensitised animals or hybridomas; and desorbed under acid or slightly denaturing conditions. In particular, recombinant Growth Hormone may be purified from *E. coli* cultures according to the method disclosed in WO

87/00204 or EP 0 177 343.

Example 3 - Fluorescence detection.

In order to detect the hGHf by fluorescent measurements in samples from a mammalian subject to whom the hGHf has been administered, it is preferable to purify or enrich the sample (i.e. blood or urine) to reduce background fluorescent interference. This can be routinely accomplished by the use of a number of standard chromatographic techniques such as HPLC, FPLC, affinity chromatography, or immunoaffinity chromatography. Fluorescence may be increased by prior denaturation of the protein, for example by use of mild heat treatment and/or chaotropic agents (e.g. 1-6M Urea or guanidimium chloride).

W-fluorescence is measurable using standard techniques such as, for example, an SLM 8000 single photon counting spectrofluorometer. The purified sample is subjected to excitation around 297nm across a 2mm cell using a mercury-Xenon arc lamp and fluorescence detected around 345nm using a Mullard XP 2020Q rapid-response photomultiplier along a 1cm path at 90^0 to the excitation beam. Scattered light is excluded by cut-off filter (Schott 310) between the sample and photomultiplier.

An alternative embodiment of the invention can be envisaged, in which exogenous hGH is provided with reduced fluorescence relative to the naturally occurring molecule, for example by replacing W at position 86 with either F or Y.

It will be appreciated that the present invention has applications in other areas such as detection of exogenous proteins over the same protein produced endogenously, for example, measuring exogenous bovine growth hormone (bGH) which is administered to increase milk or meat production in cattle. Additionally the methods of the present invention can be used to detect abuse of such anabolic proteins in humans or in animals.

It will be further appreciated that the present invention is not limited to mammalian growth hormone proteins and can be equally successfully applied to other proteins including those which are also produced endogenously and those with therapeutic applications. such as calcitonin.

Example 4 - Human Calcitonin

Calcitonin (thyrocalcitonin) is an endogenous 32 amino acid peptide hormone produced by certain cells in the thyroid gland whose principle action is to lower the levels of calcium and phosphate in the blood. It is used clinically to treat several disorders such as hypercalcaemia and bone disorders such as Paget's disease and Osteoporosis. The amino acid sequence of calcitonin is illustrated in Figure 5, and included as Seq. ID No. 4 in the attached Sequence Listing.

Calcitonin may be negatively tagged (i.e. provided with reduced fluorescence) or positively tagged (i.e. provided with reduced fluorescence) relative to the naturally occurring molecule as follows:

To reduce fluorescence: replace Y 12 with L;

To increase fluorescence any one of the following substitutions may be performed: replace Y 12 with W; replace any F residue (located at positions 16, 19, and 22) with W; replace any two F residues (located at positions 16, 19, and 22) with W, preferably F 16 and F 22 (so as to avoid possible complications of "quenching" or other interference if fluorophores are too close together); replace any F residue (located at positions 16, 19, and 22) with Y, preferably F 22; replace any two F residues (located at positions 16, 19, and 22) with Y, preferably F 16 and F 22.

Example 5 - Human Growth Hormone Releasing Factor

Human Growth Hormone Releasing Factor (HGHRF) is an endogenous 44 amino acid peptide hormone that controls the release of human growth hormone. Consequently its clinical uses are similar to those for human growth hormone itself. The amino acid sequence of HGHRF is shown in Figure 6, and included as Seq. ID No. 5 in the attached sequence listing. HGHRF may be positively tagged (i.e. provided with increased fluorescence) relative to the naturally occurring molecule, by performing any one of the following substituions: replace any one of R 41, 42, or 43 with W; or replace both R 41 and R 43 with W.

Example 6 - Human Insulin

Human insulin is an endogenous hormone produced in the pancreas by the beta cells of the islets of Langerhans and is important for regulating the amount of glucose in the blood.

Lack of this hormone gives rise to diabetes mellitus, and as such insulin is used clinically to treat this condition. Mature insulin consists of two peptides, termed A and B, which are joined by two disulphide bridges: one between A chain C7 and B chain C7; and a second between A chain C20 and B chain C19. The sequence of the A and B chains of human insulin are shown in Figures 7A and 7B respectively, and are included as Seq. ID Nos. 6 and 7 in the attached sequence listing.

Human insulin may be negatively tagged or positively tagged, relative to the naturally occurring molecule, so as to be provided with reduced or increased fluorescence respectively, as described below:

To reduce fluorescence: replace any one or more Y residues (located at positions A 14; B 16 B 26) with either L or F;

To increase fluorescence: either; replace any F residue (located at positions B 24 and B 25) with W; replace any Y residue (located at positions A 14; B 16; and B 26) and either F residue (located at positions B 24 and B 25) with W.

Example 7 - Human Erythropoietin (EPO)

Human Erythropoietin is the principal endogenous factor responsible for the regulation of red blood cell production during steady-state conditions and for accelerating recovery of red blood cell mass following haemorrhage. As a result, EPO has important clinical uses where elevated levels of red blood cell expression is indicated. The amino acid sequence of EPO is shown in Figure 8, and is included as Seq. ID No. 8 in the attached sequence listing. EPO may conveniently be negatively tagged relative to naturally occurring EPO by replacing any one or more W residues (located at positions 51, 64 and 88) with F.

Example 8 - Human Interleukin 2 (IL-2)

Human Interleukin 2 is an endogenous factor produced and secreted primarily by activated T helper cells that acts as a paracrine factor driving the expansion of antigen specific cells and as a paracrine factor influencing the activity of a number of other cells including B cells, NK cells and LAK cells. Because of this central role of the IL-2/IL-2R system in mediation of the immune response, IL-2 has important diagnostic and therapeutic implications. For

example, IL-2 has shown promise as an anti-cancer drug by virtue of its ability to stimulate the proliferation and activities of tumour-attacking LAK and TIL cells. The amino acid sequence of human IL-2 is shown in figure 9 and is included as Seq. ID No. 9 in the attached sequence listing.

Human IL-2 may conveniently be negatively tagged or positively tagged (i.e. provided with reduced or increased fluorescence, respectively) relative to naturally occurring IL-2 as follows:

To reduce fluorescence: replace W 121 with either Y or F;

To increase fluorescence: either; replace any one or more F residues (located at positions 42, 44, 78, and 103) with W; or replace any one or more Y residues (located at positions 31, 45 and 107) with W.

CLAIMS

1. A method of detecting the presence in a sample of a polypeptide exogenously administered to a mammalian subject from whom the sample is obtained, and distinguishing between such an exogenously administered polypeptide and a naturally-occurring endogenous polypeptide present in the sample; the method comprising obtaining a sample from the subject; and subjecting the sample to analysis of fluorescence at a suitable wavelength; wherein the exogenously administered polypeptide is tagged with a greater or lesser amount of fluorescence activity, relative to the untagged endogenous polypeptide, at the wavelength(s) analysed.

2. A method according to claim 1, wherein the sample is subjected to processing, prior to fluorescence analysis, to enrich or purify the exogenous and endogenous molecules in the sample.

3. A method according to claim 1 or 2, wherein the sample is subjected to processing, prior to analysis, by one or more of the following: centrifugation; HPLC; FPLC; affinity chromatography; immunoaffinity chromatography; denaturation or heat treatment.

4. A method according to any one of claims 1, 2 or 3, wherein the sample is a sample of body fluid or tissue obtained from a human or other mammalian subject.

5. A method according to any one of the preceding claims, wherein the sample comprises one or more of the following: blood; saliva; sweat; urine; semen; tears.

6. A method according to any one of the preceding claims, wherein the tagged molecule has greater fluorescence activity, at the wavelength analysed, than the untagged molecule.

7. A method according to any one of the preceding claims, wherein the tagged molecule comprises one or more fluorophores not present in the untagged molecule.

8. A method according to claim 7, wherein a compound comprising a tagging fluorophore is incorporated in the tagged molecule by means of a peptide bond.

9. A method according to claim 7 or 8, wherein the fluorophore comprises tyrosine, tryptophan or a synthetic amino acid derivative.

10. A method according to any one of the preceding claims, wherein the tagged molecule comprises a tagged therapeutic polypeptide and/or tagged hormone.

11. A method according to any one of the preceding claims, wherein the tagged molecule comprises one of the following: a tagged human, bovine or porcine growth hormone; tagged calcitonin; tagged erythropoietin; tagged growth hormone releasing factor; tagged insulin; or tagged interleukin-2.

12. A method according to any one of the preceding claims, wherein the tagged molecule comprises growth hormone tagged with a tryptophan residue at one or more of positions 10, 31, 97, 160 or 176.

13. A composition for administration to a mammalian subject, the composition comprising a polypeptide and a physiologically acceptable carrier substance, characterised in that the polypeptide is tagged with a greater or lesser amount of fluorescent activity relative to an untagged polypeptide endogenously present in the subject, the tagged molecule thereby being distinguishable from the untagged molecule by analysis of the fluorescence characteristics of the respective molecules, excluding those compositions in which the tagged molecule is Growth Hormone and wherein the fluorescent tagging consists solely of one or more of the following substitutions in the tagged Growth Hormone: G40 → Y; F52 → Y; W86 → F, Y, L, I or V; F103 → Y; and I137 → Y.

14. A composition according to claim 13, wherein the tagged molecule comprises a number of tryptophan residues different from the number of tryptophan residues present in the untagged molecule, and the tagging is effected thereby.

15. A composition according to claim 13 or 14, wherein the tagged molecule comprises two or more tryptophan residues greater than the number of tryptophan residues present in the untagged molecule.

16. A composition according to any one of claims 13, 14 or 15, wherein the tagged molecule comprises a therapeutic polypeptide and/or hormone.

17. A composition according to any one of claims 13-16, wherein the tagged molecule comprises one of the following: tagged human, bovine or porcine growth hormone; tagged

calcitonin; tagged erythropoietin; tagged growth hormone releasing factor; tagged insulin; or tagged interleukin-2.

18. A composition according to any one of claims 13-17, wherein the tagged molecule comprises growth hormone tagged with a tryptophan residue at one or more of positions 10, 31, 97, 160 or 176.

19. A tagged growth hormone comprising a tryptophan residue substituted for a phenylalanine residue present in a naturally-occurring growth hormone molecule.

20. A tagged growth hormone comprising a tryptophan residue at one or more of positions 10, 31, 97, 160 or 176.

21. A tagged growth hormone comprising a tryptophan residue at position 31 and/or 97.

22. A nucleic acid sequence encoding a tagged growth hormone in accordance with any one of claims 19, or 21.

23. A nucleic acid expression construct comprising a nucleic acid sequence in accordance with claim 22.

24. A nucleic acid sequence comprising nucleotides 114-695 of the nucleic acid sequence shown in Figure 2.

25. A method substantially as hereinbefore defined.

26. A composition substantially as hereinbefore defined.

Fig.1.

```
F P T I P L S R L F D N A M L R A H R L H Q L A F  25
D T Y Q E F E E A Y I P K E Q K Y S F L Q N P Q T  50
S L C F S E S I P T P S N R E E T Q Q K S N L E L  75
L R I S L L L I Q S W L E P V Q F L R S V F A N S 100
L V Y G A S D S N V Y D L L K D L E E G I Q T L M 125
G R L E D G S P R T G Q I P K Q T Y S K F D T N S 150
H N D D A L L K N Y G L L Y C F R K D M D K V E T 175
F L R I V Q C R S V E G S C G F                   191
```

Fig.2.

```
                                          vNde 1
GGATCCTTTTGTTTAACTTTAAGAAGGAGATATACAT ATG CGT CCG  47
TCT ATC CAC CGT ACC GCT ATC GCT GCT GTT CTG GCT ACC  86
GCT TTC GTT GCT GGT ACC GCT CTG GCA TTC CCG ACC ATC 125
CCG CTG TCT CGT CTG TTC GAC AAC GCT ATG CTG CGT GCT 164
CAC CGT CTG CAC CAG CTG GCT TTC GAC ACC TAC CAG GAA 203
TGG GAA GAA GCT TAC ATC CCG AAA GAA CAG AAA TAC TCT 242
TTC CTG CAG AAC CCG CAG ACC TCT CTG TGC TTC TCT GAA 281
TCT ATC CCG ACC CCG TCT AAC CGT GAA GAA ACC CAG CAG 320
AAA TCT AAC CTG GAA CTG CTG CGT ATC TCT CTG CTG CTG 359
ATC CAG TCT TGG CTG GAA CCG GTT CAG TTC CTG CGT TCT 398
GTT TGG GCT AAC TCT CTG GTT TAC GGT GCT TCT GAC TCT 437
AAC GTT TAC GAC CTG CTG AAA GAC CTG GAA GAA GGT ATC 476
CAG ACC CTG ATG GGT CGT CTG GAA GAC GGT TCT CCG CGT 515
ACC GGT CAG ATC TTC AAA CAG ACC TAC TCT AAA TTC GAC 554
ACC AAC TCT CAC AAC GAC GAC GCT CTG CTG AAA AAC TAC 593
GGT CTG CTG TAC TGC TTC CGT AAA GAC ATG GAC AAA GTT 632
GAA ACC TTC CTG CGT ATC GTT CAG TGC CGT TCT GTT GAA 671
                          v Xho 1
GGT TCT TGC GGT TTC TAA CTC GAG                     695
```

Fig.4.

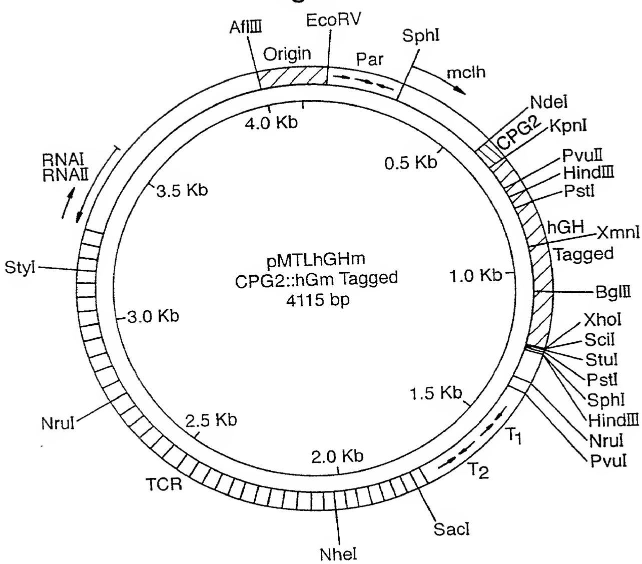
AflIII
EcoRV
Origin
Par
SphI
mclh
NdeI
CPG2
KpnI
PvuII
HindIII
PstI
hGH
XmnI
Tagged
BglII
XhoI
SciI
StuI
PstI
SphI
HindIII
NruI
PvuI
T1
T2
SacI
NheI
TCR
NruI
StyI
RNAI
RNAII
4.0 Kb
0.5 Kb
3.5 Kb
1.0 Kb
3.0 Kb
1.5 Kb
2.5 Kb
2.0 Kb
pMTLhGHm
CPG2::hGm Tagged
4115 bp

Fig.3.

F P T I P L S R L F D N A M L R A H R L H Q L A F 25
D T Y Q E **W** E E A Y I P K E Q K Y S F L Q N P Q T 50
S L C F S E S I P T P S N R E E T Q Q K S N L E L 75
L R I S L L L I Q S W L E P V Q F L R S V **W** A N S 100
L V Y G A S D S N V Y D L L K D L E E G I Q T L M 125
G R L E D G S P R T G Q I P K Q T Y S K F D T N S 150
H N D D A L L K N Y G L L Y C F R K D M D K V E T 175
F L R I V Q C R S V E G S C G F 191

Fig.5.

C G N L S T C M L G T Y T Q D F N K F H T F P Q T 25
A I G V G A P-NH_2 32

Fig.6.

Y A D A I F T N S Y R K V L G Q L S A R K L L Q D 25
I M S R Q Q G E S N Q E R G A R R R L-NH_2 44

Fig.7A.

Chain A

G I V E Q C C T S I C S L Y Q L E N Y C N 21

Fig.7B.

Chain B

F V N Q H L C G S H L V E A L Y L V C G E R G F F 25
Y T P K T

Fig.8.

A P P R L I C D S R V L Q R Y L L E A K E A E N I 25
T T G C A E H C S L N E N I T V P D T K V N F Y A 50
W K R M E V G Q Q A V E V W Q G L A L L S E A V L 75
R G Q A L L V N S S Q P W E P L Q L H V D K A V S 100
G L R S L T T L L R A L G A Q K E A I S P P D A A 125
S A A P L R T I T A D T F R K L F R V Y S N F L R 150
G K L K L Y T G E A C R T G D 165

Fig.9.

A P T S S S T K K T Q L Q L E H L L L D L Q M I L 25
N G I N N Y K N P K L T R M L T F K F Y M P K K A 50
T E L K H L Q C L E E E L K P L E E V L N L A Q S 75
K N F H L R P R D L I S N I N V I V L E L K G S E 100
T T F M C E Y A D E T A T I V E F L N R W I T F C 125
Q S I I S T L T 133

FOR UTILITY/DESIGN CIP/PCT NATIONAL/PLANT ORIGINAL/SUBSTITUTE/SUPPLEMENTAL DECLARATIONS	RULE 63 (37 C.F.R. 1.63) DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION IN THE UNITED STATES PATENT AND TRADEMARK OFFICE	PM & S FORM

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name, and I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the **INVENTION ENTITLED** Improvements in or Relating to Detection of Molecules in Samples

the specification of which (CHECK applicable BOX(ES))

X BOX(ES)
- → A. ☐ is attached hereto.
- → B. ☐ was filed on ______ as U.S. Application No. ______ / ______
- → C. ☒ was filed as PCT International Application No. PCT/ GB98 /03449 on 16th November 1998

and (if applicable to U.S. or PCT application) was amended on ______

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose all information known to me to be material to patentability as defined in 37 C.F.R. 1.56. I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT International Application which designated at least one other country than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate, or PCT International Application, filed by me or my assignee disclosing the subject matter claimed in this application and having a filing date (1) before that of the application on which priority is claimed, or (2) if no priority claimed, before the filing date of this application:

PRIOR FOREIGN APPLICATION(S)

Number	Country	Day/MONTH/Year Filed	Date first Laid-open or Published	Date Patented or Granted	Priority Claimed Yes	Priority Claimed No
9723955.2	GB	14 NOVEMBER 1997			X	

I hereby claim domestic priority benefit under 35 U.S.C. 119(e) or 120 and 365(c) of the indicated United States applications listed below and PCT international applications listed above or below and, if this is a continuation-in-part (CIP) application, insofar as the subject matter disclosed and claimed in this application is in addition to that disclosed in such prior applications, I acknowledge the duty to disclose all information known to me to be material to patentability as defined in 37 C.F.R. 1.56 which became available between the filing date of each such prior application and the national or PCT international filing date of this application:

PRIOR U.S. PROVISIONAL, NONPROVISIONAL AND/OR PCT APPLICATION(S)

Application No. (series code/serial no.)	Day/MONTH/Year Filed	Status pending, abandoned, patented	Priority Claimed Yes	Priority Claimed No

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

And I hereby appoint Pillsbury Madison & Sutro LLP, Intellectual Property Group, 1100 New York Avenue, N.W., Ninth Floor, East Tower, Washington, D.C. 20005-3918, telephone number (202) 861-3000 (to whom all communications are to be directed), and the below-named persons (of the same address) individually and collectively my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith and with the resulting patent, and I hereby authorize them to delete names/numbers below of persons no longer with their firm and to act and rely on instructions from and communicate directly with the person/assignee/attorney/firm/ organization who/which first sends/sent this case to them and by whom/which I hereby declare that I have consented after full disclosure to be represented unless/until I instruct the above Firm and/or a below attorney in writing to the contrary.

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Carl G. Love	18781	Kendrew H. Colton	30368	Ruth N. Morduch	31044	Adam R. Hess	41835
Kevin E. Joyce	20508	G. Paul Edgell	24238	Richard H. Zaitlen	27248		
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(FOR ADDITIONAL INVENTORS, check box ☐ to attach PAT 116-2 same information for each re signature, name, date, citizenship, residence and address.)

09/554451
526 Rec'd PCT/PTO 15 MAY 2000

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: Improvements in or Relating to Detection of Molecules in Samples

(iii) NUMBER OF SEQUENCES: 9

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 191 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
Phe Pro Thr Ile Pro Leu Ser Arg Leu Phe Asp Asn Ala Met Leu Arg
1               5                   10                  15

Ala His Arg Leu His Gln Leu Ala Phe Asp Thr Tyr Gln Glu Phe Glu
            20                  25                  30

Glu Ala Tyr Ile Pro Lys Glu Gln Lys Tyr Ser Phe Leu Gln Asn Pro
        35                  40                  45

Gln Thr Ser Leu Cys Phe Ser Glu Ser Ile Pro Thr Pro Ser Asn Arg
    50                  55                  60

Glu Glu Thr Gln Gln Lys Ser Asn Leu Glu Leu Leu Arg Ile Ser Leu
65                  70                  75                  80

Leu Leu Ile Gln Ser Trp Leu Glu Pro Val Gln Phe Leu Arg Ser Val
                85                  90                  95

Phe Ala Asn Ser Leu Val Tyr Gly Ala Ser Asp Ser Asn Val Tyr Asp
```

100 105 110

Leu Leu Lys Asp Leu Glu Glu Gly Ile Gln Thr Leu Met Gly Arg Leu
115 120 125

Glu Asp Gly Ser Pro Arg Thr Gly Gln Ile Pro Lys Gln Thr Tyr Ser
130 135 140

Lys Phe Asp Thr Asn Ser His Asn Asp Asp Ala Leu Leu Lys Asn Tyr
145 150 155 160

Gly Leu Leu Tyr Cys Phe Arg Lys Asp Met Asp Lys Val Glu Thr Phe
165 170 175

Leu Arg Ile Val Gln Cys Arg Ser Val Glu Gly Ser Cys Gly Phe
180 185 190

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 695 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GGATCCTTTT TGTTTAACTT TAAGAAGGAG ATATACATAT GCGTCCGTCT ATCCACCGTA 60

CCGCTATCGC TGCTGTTCTG GCTACCGCTT TCGTTGCTGG TACCGCTCTG GCATTCCCGA 120

CCATCCCGCT GTCTCGTCTG TTCGACAACG CTATGCTGCG TGCTCACCGT CTGCACCAGC 180

TGGCTTTCGA CACCTACCAG GAATGGGAAG AAGCTTACAT CCCGAAAGAA CAGAAATACT 240

CTTTCCTGCA GAACCCGCAG ACCTCTCTGT GCTTCTCTGA ATCTATCCCG ACCCCGTCTA 300

ACCGTGAAGA AACCCAGCAG AAATCTAACC TGGAACTGCT GCGTATCTCT CTGCTGCTGA 360

TCCAGTCTTG GCTGGAACCG GTTCAGTTCC TGCGTTCTGT TTGGGCTAAC TCTCTGGTTT 420

ACGGTGCTTC TGACTCTAAC GTTTACGACC TGCTGAAAGA CCTGGAAGAA GGTATCCAGA 480

CCCTGATGGG TCGTCTGGAA GACGGTTCTC CGCGTACCGG TCAGATCTTC AAACAGACCT 540

ACTCTAAATT CGACACCAAC TCTCACAACG ACGACGCTCT GCTGAAAAAC TACGGTCTGC 600

TGTACTGCTT CCGTAAAGAC ATGGACAAAG TTGAAACCTT CCTGCGTATC GTTCAGTGCC 660

GTTCTGTTGA AGGTTCTTGC GGTTTCTAAC TCGAG 695

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 191 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
Phe Pro Thr Ile Pro Leu Ser Arg Leu Phe Asp Asn Ala Met Leu Arg
1               5                   10                  15

Ala His Arg Leu His Gln Leu Ala Phe Asp Thr Tyr Gln Glu Trp Glu
            20                  25                  30

Glu Ala Tyr Ile Pro Lys Glu Gln Lys Tyr Ser Phe Leu Gln Asn Pro
        35                  40                  45

Gln Thr Ser Leu Cys Phe Ser Glu Ser Ile Pro Thr Pro Ser Asn Arg
    50                  55                  60

Glu Glu Thr Gln Gln Lys Ser Asn Leu Glu Leu Leu Arg Ile Ser Leu
65                  70                  75                  80

Leu Leu Ile Gln Ser Trp Leu Glu Pro Val Gln Phe Leu Arg Ser Val
                85                  90                  95

Trp Ala Asn Ser Leu Val Tyr Gly Ala Ser Asp Ser Asn Val Tyr Asp
            100                 105                 110

Leu Leu Lys Asp Leu Glu Glu Gly Ile Gln Thr Leu Met Gly Arg Leu
        115                 120                 125

Glu Asp Gly Ser Pro Arg Thr Gly Gln Ile Pro Lys Gln Thr Tyr Ser
    130                 135                 140

Lys Phe Asp Thr Asn Ser His Asn Asp Asp Ala Leu Leu Lys Asn Tyr
145                 150                 155                 160

Gly Leu Leu Tyr Cys Phe Arg Lys Asp Met Asp Lys Val Glu Thr Phe
                165                 170                 175

Leu Arg Ile Val Gln Cys Arg Ser Val Glu Gly Ser Cys Gly Phe
            180                 185                 190
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Cys Gly Asn Leu Ser Thr Cys Met Leu Gly Thr Tyr Thr Gln Asp Phe
1   5   10   15

Asn Lys Phe His Thr Phe Pro Gln Thr Ala Ile Gly Val Gly Ala Pro
            20   25   30

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1   5   10   15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg Gln Gln Gly
            20   25   30

Glu Ser Asn Gln Glu Arg Gly Ala Arg Arg Arg Leu
        35   40

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu
1   5   10   15

Glu Asn Tyr Cys Asn
            20

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```
Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
1               5                   10                  15

Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Thr
            20                  25                  30
```

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 165 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
Ala Pro Pro Arg Leu Ile Cys Asp Ser Arg Val Leu Gln Arg Tyr Leu
1               5                   10                  15

Leu Glu Ala Lys Glu Ala Glu Asn Ile Thr Thr Gly Cys Ala Glu His
            20                  25                  30

Cys Ser Leu Asn Glu Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe
        35                  40                  45

Tyr Ala Trp Lys Arg Met Glu Val Gly Gln Gln Ala Val Glu Val Trp
    50                  55                  60

Gln Gly Leu Ala Leu Leu Ser Glu Ala Val Leu Arg Gly Gln Ala Leu
65                  70                  75                  80

Leu Val Asn Ser Ser Gln Pro Trp Glu Pro Leu Gln Leu His Val Asp
                85                  90                  95

Lys Ala Val Ser Gly Leu Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu
            100                 105                 110

Gly Ala Gln Lys Glu Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala
        115                 120                 125

Pro Leu Arg Thr Ile Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg Val
    130                 135                 140

Tyr Ser Asn Phe Leu Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala
145                 150                 155                 160

Cys Arg Thr Gly Asp
                165
```

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 133 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His
1               5                   10                  15

Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys
            20                  25                  30

Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe Tyr Met Pro Lys
        35                  40                  45

Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys
    50                  55                  60

Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu
65                  70                  75                  80

Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu
                85                  90                  95

Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala
            100                 105                 110

Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Cys Gln Ser Ile
        115                 120                 125

Ile Ser Thr Leu Thr
    130
```

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